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On the Use of the R_{50}

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ABSTRACT: R_{50} is a statistic used to describe the repellency of a substance in the manner that an LC_{50} is used to describe toxicity. Although the R_{50} is calculated using the same methods as for estimating an LC_{50}, the data initially collected on individual animals are proportional, not binary. Transforming nonbinary data into binary data is subjective, can result in substantial losses of information, and, consequently, can result in a greater chance for erroneous inferences concerning the repellency of a substance. The subjective nature of R_{50} estimation arises because a test animal is defined as repelled if less than 50% of treated food items are consumed. The R_{50} is calculated as the concentration at which 50% of the population consumes less than 50% of the food times. It is more appropriate and of more direct interest to estimate the concentration of a substance at which an average of 50% of the food items are consumed rather than to estimate the R_{50}.

Problematic aspects of R_{50} estimation are demonstrated in this paper, and alternative analyses are recommended for studying the repellency of a substance. Example data sets are used to compare inferences from estimation of an R_{50} versus use of inverse regression methods to estimate concentrations at which various proportions of food items are consumed.

KEY WORDS: R_{50}, repellency, inverse regression

The lethality of a chemical toxic to an animal species is usually described by conducting a bioassay experiment and estimating the median lethal dose or concentration (that is, the dose at which one half of the population would be expected to die—LD_{50} or LC_{50}). The data collected for estimating an LD_{50} are binary (that is, each animal is recorded as alive or dead—0 or 1). Similarly, a natural parameter for describing a chemical's ability to repel an animal species from a food source is the median repellent concentration (R_{50}), the concentration at which one half of the population would be repelled. However, unlike lethality, the concept of repellency is not well defined. In practice, determination of an individual's repellency has required an arbitrary definition based on the amount of a food item eaten. Thus, a continuous response, such as amount or proportion of food eaten, is reduced to a binary score based on the operant definition. These data are then subjected to analyses for estimating the R_{50}. In this paper, we demonstrate that reducing nonbinary data to binary can result in: losses of information, the need for larger sample sizes, incorrect inferences about the chemical's repellent qualities, and less direct descriptions of the chemical's ability to protect a crop. Alternative analyses are suggested.

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The R_{50} Experiment

We give a general outline of experiments designed to estimate an R_{50}. Additional information on these experimental protocols can be found in Refs *1* and *2*. An early reference involving the R_{50} describes the use of preconditioning tests [*3*]. Untreated food particles are used to condition animals for testing and to determine the amounts of food used for testing. Animals are in some fashion randomly assigned to treatment groups (preferably such that all weight classes are represented in each treatment group). Each treatment group is designated to receive food treated with a particular concentration of the test chemical. Each individual (housed separately) in each group receives food particles treated at that group's level. Usually this means each individual receives an odd number of treated food particles, such as 25 seeds. Animals that consume less than half of the food offered (for example, 12 seeds or less) are considered repelled. Their binary measurement would be 1. Animals that consume more than half are considered not repelled, and their binary measurement would be 0.

To illustrate, consider the artificial data set in Table 1. The estimated R_{50}, using probit analysis (see, for example, Refs *4* and *5*), is 87.7 with a 95% confidence interval of 23.6 to 126.6. The R_{90} (concentration at which 90% of the population would be expected to be repelled) is 205.9 with 95% confidence limits of 160.7, 326.3. We also use these data to demonstrate alternative analytical methods and their associated inferences.

Effects of Redefining Repellency

Suppose that one does not wish to consider an animal as repelled if it will eat almost 50% of the treated food presented to it. The animal may be defined as repelled, but the food that the chemical is supposed to protect may be half gone. If we redefine repelled as eating less than 25% (six particles or less in our example) of the food particles eaten, then no animal in Table 1 would be considered repelled and, of course, an R_{50} could not be calculated.

This example demonstrates the ambiguities associated with defining repellency as a binary variable. Another data set with the same R_{50} as our data when repellency is defined as less than 50% consumption would not necessarily have the same R_{50} as our data if repellency is redefined as less than 25% consumption. The R_{50} is supposed to represent the concentration of the chemical at which 50% of the animal population is repelled, but an animal can eat 49.9% of the treated food and be considered repelled. Thus, at the R_{50}, nearly 50% of the treated food presented could be consumed by the half of the population that is repelled and 100% could be consumed by the half that is not repelled.

TABLE 1—*Example data of the number of particles eaten when 25 food particles are presented.*

Percent Concentration	Animal (Within Concentration)[a]										Mean Percent	
	1	2	3	4	5	6	7	8	9	10	Consumed, %	Repelled, %
30	20	21	25	11	22	12	23	22	12	24	78	30
90	22	12	11	22	21	22	10	12	24	9	66	50
150	18	10	11	12	21	11	12	22	12	11	56	70
210	12	10	11	9	14	12	11	12	9	12	45	90
270	10	8	9	9	7	10	8	11	7	9	35	100

[a] A different set of ten animals is used at each concentration.

Defining repellency is ambiguous, and the concept of the R_{50} is confusing. Because the purpose of a repellent chemical is to protect a crop from being eaten, a more realistic concept would be to study the amount of food consumption directly. The concentration at which 50% of the food would be expected to be consumed could be estimated (and named the FC_{50}). This parameter would relate more directly to a repellent chemical's ability to protect a crop. We consider this approach in later sections.

Loss of Information

When a transformation that is not one to one is applied to data, a loss of information occurs. The fidelity of the transformed data is less than the original data. Transforming proportional data into binary data is an example that is not one to one; that is, each original data value is not represented by a unique transformed value.

Consider, as an example, the repellent study in Table 1. Each animal is offered 25 food particles. If 12 or less are eaten the animal is considered repelled and assigned the value 1. If 13 or more are eaten, the animal is not considered repelled and assigned the value 0. Thus, for example, the 2 animals that consume 1 and 10 seeds, respectively, receive the same repellency score of 1, even though the practical implications of these 2 values may be very different. Obviously, information concerning the repellent property of the chemical is being lost.

Sample Size

The use of a transformation that diminishes the information contained in the data requires more data to make up for the loss. This problem is further compounded because the estimation procedures to be applied to the transformed binary data perform poorly at small sample sizes [*4,5*]. At the smaller sample sizes (for example, less than 30 total animals, less than 5 concentrations, or less than 6 animals per concentration), the probit and logit estimation methods may not be able to calculate an estimate or the associated confidence limits or both. Although Thompson's moving average method [*6*] has traditionally been used to calculate approximate estimates in these situations, its confidence limits are not accurate [*4*].

Consider again the data in Table 1. Suppose only ten animals were available for this study, for example, the first two animals at each concentration. The number of animals out of two repelled at each concentration is now 0, 1, 1, 2, 2, respectively, for the concentrations 30, 90, 150, 210, 270. The estimate of the R_{50} from this reduced data set is 119.7, but finite confidence limits could not be calculated.

Alternate Approach to R_{50} Estimation

We now consider using the proportional data from a repellency experiment, without an arbitrary definition of repellency that leads to a loss of information. Such data can be more efficiently analyzed using linear regression techniques. The proportion of food particles consumed (or, equivalently, the number of food particles consumed if all animals are presented the same number) is treated as the response variable (y) that can be related to the concentration of the chemical applied to the food (x). Inverse regression methods (predicting concentration, x, from consumption, y) could be used to estimate the concentration at which 50% consumption would be expected (FC_{50}) [*7,8*].

There are many advantages to this approach. No information is lost from applying a binary transformation to the data. Interpretation of the results relates directly to how well a concentration of a chemical relates to protection of the food particles from consumption. There is no reliance on an arbitrary definition of repellency. Regression methods will produce valid estimates at smaller sample sizes than the bioassay methods. Thus, if only a few animals are available, a valid experiment may still be conducted. Also, for a fixed number of animals, more concentrations of the chemical (values of x) may be studied by using fewer animals per concentration. Trying this in the bioassay (R_{50}) format risks producing data from which valid estimates and confidence intervals cannot be calculated. Inverse regression methods allow the estimation of the concentration level with confidence limits where the expected consumption is 50% (see, for example, Refs *7* and *8*). This relates directly to protecting the food. Repelling 50% of a population by limiting that 50% of the population to less than 50% food consumption is confusing and a less direct measure of food protection.

Example 1

Again we consider the data in Table 1. The number of food particles consumed is related to the chemical concentration by the equation

$$y = 20.465 - 0.0435x \tag{1}$$

where y is the number of particles consumed and x is the concentration. The concentration at which 50% of the food particles are consumed is 183.1 with 95% confidence limits of 156.0, 217.7 (or width of 61.7 versus a width of 103 for the R_{50} interval). Recall that the estimated R_{50} was 87.7, which is less than half the concentration at which 50% of the food is protected. This indicates that, at the R_{50}, 50% of the animals may be repelled (according to the arbitrary definition of repellency) but much more than 50% of the food may be lost. Note that at the estimated R_{50} concentration approximately two thirds of the food is consumed.

If we now consider only the data from the first two animals in each group (as we did for the R_{50} estimation), the following equation is estimated for relating number of particles consumed to the concentration of the chemical:

$$y = 21.55 - 0.0483x \tag{2}$$

Note how similar this equation is to Eq 1 where all data were used. Equation 2 estimates the concentration at which 50% of the food particles are consumed to be 187.2 (which is very close to the 183.1 predicted when using all data). The 95% confidence limits are 136.6, 277.7. The width of these limits is more than twice that of the confidence interval as when all data were used. However, in contrast to R_{50} estimation with the same two animals per concentration, these limits are calculable. It is possible that, had we used different sets of two animals per concentration, a less accurate estimate of 50% consumption level and a wider 95% confidence interval would have resulted. However, it is unlikely that an R_{50} with confidence limits could have been calculated using any subset of Table 1 where there were two animals per concentration. These results indicate that acceptable results can be achieved with much less data for the regression methods than for the bioassay methods. For a fixed number of animals, more concentrations can be studied using the regression methods than the bioassay methods.

Example 2

We now consider repellency data originally given in Ref *9* and later published in Ref *10*. In this study, four concentrations of methiocarb are considered for repelling rock doves.

TABLE 2—*Three percent consumption data scenarios resulting in identical binary data.*

	Concentration																			
Scenario	0.056					0.100					0.178					0.312				
1	50.5	52	51	53.5	53	50.5	51	52	50.5	11	51	53	27	20	34	1.5	1	1	3.5	3
2	96.5	98	97	99.5	99	49	76.5	84	89	96.5	51	49	48	53	49	1	4	1.5	1.5	2
3	53	51	50.5	52.5	53	52	53	53.5	49.5	52	49	47	52	48.5	53	47.5	47	47	49.5	49
Number Repelled	0					1					3					5				

Birds in this study were considered repelled if they ate less than 50% by weight of the food presented to them. Five birds were tested at each concentration of methiocarb. The number repelled at the 0.056, 0.100, 0.178, and 0.312% concentration levels were 0, 1, 3, and 5, respectively. An R_{50} estimate of 0.15 with 95% confidence limits of 0.10, 0.24 was given. However, the values were estimated using Thompson's moving average [*6*]. Because of the problems noted earlier associated with this estimation procedure at small sample sizes, we recalculated the R_{50} and confidence limits using probit analysis. The resulting R_{50} estimate was 0.16 with 95% confidence limits of 0.11 and 0.31. We will only consider these latter values for discussion.

These binary data could have been arrived at from an infinite number of possibilities of actual proportional consumption. In Table 2, we present three scenarios, all of which result in binary repellency data identical to that given above. The estimated levels where 50% food consumption would occur (and the associated 95% confidence limits) are, respectively, for Scenarios 1, 2, and 3: 0.075 (0.016, 0.108), 0.18, (0.17, 0.19), and 0.19 (0.14, 0.25).

The first value is about half of the estimated R_{50}. This illustrates the situation in which the R_{50} could imply overuse of the chemical, that is, the R_{50} indicates the use of higher concentrations of the chemical than necessary to protect the crop. The 50% consumption level is slightly higher than the R_{50} (12.5 and 18.8%, respectively) for the second and third data sets. This implies that in each of these cases the R_{50} level could imply better protection of the food than actually occurs.

The important point to draw from the data in Table 2 is that three very distinct consumption data sets each lead to the identical binary data set from which the R_{50} is estimated. The data from Scenario 1 indicate significantly greater protection from consumption than the data in Scenarios 2 and 3. Scenario 3 implies little concentration effect in the range the chemical was applied (all consumption is near 50%), whereas Scenario 2 has a much larger response to concentration (a steeper slope of the regression line).

Conclusions

The purpose of a repellent chemical is to prevent the food item on which it is applied from being eaten by an animal. The measurement of the chemical's efficacy is the amount of that food eaten by an animal. These are not binary measurements such as those resulting from lethality experiments with the objective of estimating an LD_{50} or an LC_{50}, and the response being measured is not just the direct effect the chemical has on the animal. The goal of protecting a crop by applying a repellent can only be described if crop protection is measured and analyzed. Laboratory experiments are conducted to provide an index as to how well a particular chemical may approach this goal. Use of the R_{50} does not approach this goal directly and can provide misleading inferences toward that goal.

The R_{50} is a confusing concept. Finding an estimate of the concentration of a chemical at which 50% of the animals eat less than 50% of a food does not directly address how well a food source is protected. By this definition, at the level at which the chemical repels 100% of the test animals, each animal could be consuming 50% of the food presented to them. We have demonstrated methods that require less data, do not lose information through a nonunique transformation of the data, and more reliably produce estimates and confidence intervals that also directly describe the protection provided by the test chemical.

Although the examples and discussion in this paper demonstrate the potential for misleading inference concerning a chemical's repellent properties, it is quite possible in many cases that the R_{50} concentration would be very close to the inverse regression estimate of the level where 50% of the food source is protected. However, to check this for a particular data set would require doing the regression calculations anyhow. In light of the definitional (for example, something other than 50% consumption by each individual), analytical (wide or incalculable confidence intervals), and conceptual problems associated with the R_{50}, there would seem to be little reason to calculate the R_{50} in addition to (or instead of) the regression estimates. We feel that application of inverse regression methods and estimation of a parameter such as the 50% food consumption level (FC_{50}) more directly approaches the objective of interest, that of indexing a chemical's repellent properties for preventing the consumption of a crop.

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